(S4) NADH dehydrogenases symposium lecture abstracts

S4/1 Structural aspects of the NADH-driven Na^+ pump (Na^+ -NQR) from *Vibrio cholerae*, and more Na^+ pumping by complex I

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During respiration, electrogenic NADH dehydrogenases couple the oxidation of NADH and reduction of quinone to the generation of electrochemical H^+ or Na^+ gradients. The two types of respiratory NADH dehydrogenases are the Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) found in some bacteria, and homologs of the mitochondrial complex I. In the Na^+-NQR from *Vibrio cholerae*, initial oxidation of NADH is catalyzed by the FAD domain of subunit NqrF. The structure of the FAD domain with NADH bound in the active site is presented, and implications for the mechanism of initial hydride transfer from NADH to FAD are discussed. We previously reported that complex I from enterobacteria translocates Na^+ rather than H^+, raising the question whether mitochondrial complex I also uses Na^+ as a coupling ion. Oxidation of NADH by submitochondrial particles from the yeast *Yarrowia lipolytica* was coupled to protonophore-resistant Na^+ uptake, indicating that a primary Na^+ pump is operative in the inner mitochondrial membrane. A rotenone-sensitive, respiratory NADH dehydrogenase was enriched and reconstituted into proteoliposomes which catalyzed NADH-dependent reduction of ubiquinone (1.4 μmol min^{-1} mg^{-1}) and Na^+ translocation (2.0 μmol min^{-1} mg^{-1}). We conclude that mitochondria from *Y. lipolytica* contain a NADH-driven Na^+ pump and propose that it represents the complex I of the respiratory chain.

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S4/2 Were there any "misassignments" among iron–sulfur clusters N4, N5 and N6b in NADH–quinone oxidoreductase (Complex I)?

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Mitochondrial NADH–quinone oxidoreductase (Complex I) is the largest energy transducing complex so far known. Bacterial complex I is simpler and smaller, but the essential redox components and the basic mechanisms of electron transfer/proton translocation are the same. Over the past four decades, Ohnishi and her collaborators have pursued extensive EPR studies at near liquid helium temperature and characterized most of the redox centers in complex I. Recently, Hirst's group challenged Ohnishi's group and published (PNAS, 2007) claiming that we misassigned EPR signals arising from cluster N5 to cluster N4, and signals from cluster N6b in the NuoI subunit to cluster N4 in the NuoG. They also proposed that cluster N5 has (4Cys)-ligands. We clearly detected, for the first time, cluster N5 signal in *E. coli* NuoCDEFG subcomplex (around 3 K, >5 mW). Based on our accumulated previous data and recent site-directed mutagenesis results, we confirmed that cluster N5 has (1His+3Cys)-ligands, as we predicted. Hirst's group could not detect EPR signals from *E. coli* N5 under any of their conditions (>5K, 0.1 mW). As to the claim of "misassignment" between clusters N4 and N6b, that was not a possibility because our mutagenesis systems did not contain cluster N6b. Therefore, we believe that we have not made any "misassignment" in our work.

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S4/3 Structural studies on bacterial complex I

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Respiratory complex I plays a central role in cellular energy production and its dysfunction is implicated in many human neurodegenerative diseases. We use bacterial complex I as a "minimal" model of the human enzyme. We have recently solved the crystal structure of the hydrophilic domain (peripheral arm) of complex I from *Thermus thermophilus*. The subcomplex consists of eight different subunits and contains all the redox centres of the enzyme, including nine iron–sulphur clusters. Seven of the clusters are arranged in the main redox pathway linking the NADH and quinone binding sites. Our electron crystallography studies revealed the organisation of about 60 transmembrane helices in the membrane arm of the complex. Our current efforts include solving the X-ray structures of the peripheral arm with bound nucleotides, as well as determination of the structure of the complete enzyme by cryo-electron microscopy and single particle analysis. Biochemical assays are used to evaluate how interactions between subunits are effected by substrates. Our latest results in these areas and implications for the mechanism of coupling between electron transport and proton translocation in complex I will be discussed.

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S4/4 Mammalian complex I: A regulable and vulnerable pacemaker in mitochondrial respiratory function

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This contribution covers regulatory features of the complex I of mammalian mitochondria. Observations on the impact of the rate of electron delivery by NAD-linked dehydrogenase on the H^+/e^- pumping ratio at the first coupling site of the respiratory chain and a Q-gated proton pump model of complex I are presented. In a variety of mitotic cell-line cultures activation in vivo, of the cAMP cascade, or direct addition of cAMP, promotes the NADH–ubiquinone oxidoreductase activity of complex I and lowers the cellular level of ROS. These effects of cAMP are found to be associated with PKA mediated serine phosphorylation in the conserved C-terminus of the subunit of complex I encoded by the nuclear gene NDUFS4. PKA mediated phosphorylation of this Ser in the C-terminus of the protein promotes its mitochondrial import and maturation. An extensive mass-spectrometry analysis of phosphorylation of complex I subunits is presented.

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